IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AFFYMETRIX, INC., a Delaware corporation, Plaintiff/Counter-Defendant, v. **ILLUMINA, INC.**, a Delaware corporation, Defendant/Counter-Plaintiff.	Civil Action No.: 04-901 JJF **PUBLIC VERSION**

APPENDIX TO ILLUMINA, INC.'S OPENING *MARKMAN* BRIEF
VOLUME 2 OF 3

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EXHIBIT S

US005143854A

United States Patent [19]

Pirrung et al.

[11] Patent Number: 5,143,854

[45] Date of Patent: Sep. 1, 1992

[54] **LARGE SCALE PHOTOLITHOGRAPHIC SOLID PHASE SYNTHESIS OF POLYPEPTIDES AND RECEPTOR BINDING SCREENING THEREOF**

[75] Inventors: **Michael C. Pirrung, Durham, N.C.; J. Leighton Read; Stephen P. A. Fodor, both of Palo Alto, Calif.; Lubert Stryer, Stanford, Calif.**

[73] Assignee: **Affymax Technologies N.V., Curacao, Netherlands Antilles**

[21] Appl. No.: **492,462**

[22] Filed: **Mar. 7, 1990**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 362,901, Jun. 7, 1989, abandoned.

[51] Int. Cl.[5] G01N 33/543; C07K 1/04; B01J 19/00

[52] U.S. Cl. 436/518; 436/528; 436/527; 436/807; 435/7.92; 435/7.94; 435/7.95; 435/961; 435/968; 435/973; 530/333; 530/334; 530/335; 530/336; 530/337

[58] Field of Search 436/518, 527, 807, 528; 530/333–335, 336–337; 435/7.92, 7.94, 7.95, 961, 968, 973

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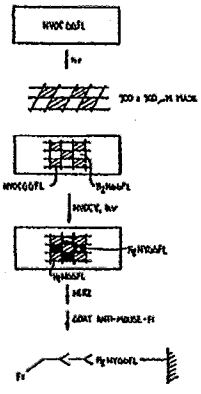
Attorney, Agent, or Firm—Kenneth J. Nussbacher; Kevin R. Kaster; Vern Norviel

[57] **ABSTRACT**

Polypeptide arrays can be synthesized on a substrate by attaching photoremovable groups to the surface of a substrate, exposing selected regions of the substrate to light to activate those regions, attaching an amino acid monomer with a photoremovable group to the activated regions, and repeating the steps of activation and attachment until polypeptides of the desired length and sequences are synthesized. The resulting array can be used to determine which peptides on the array can bind to a receptor.

13 Claims, 20 Drawing Sheets



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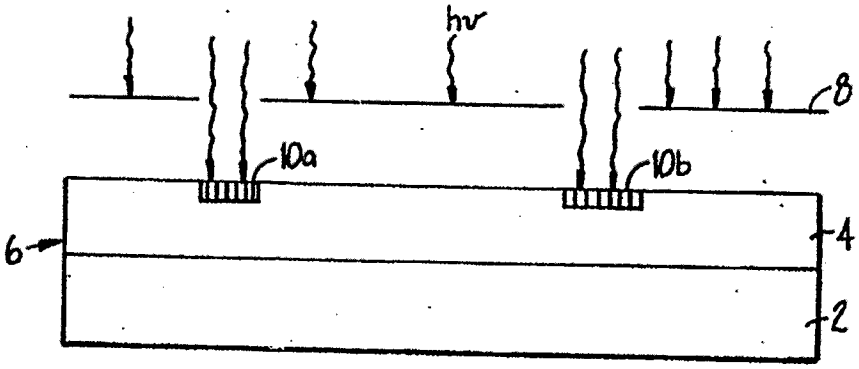

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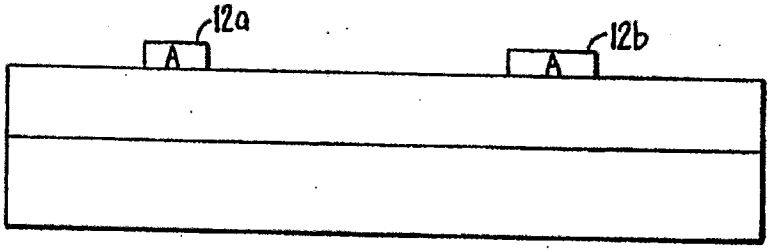
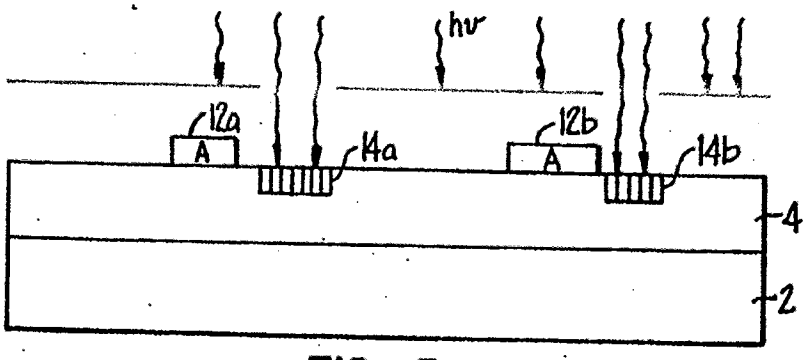


FIG._2.



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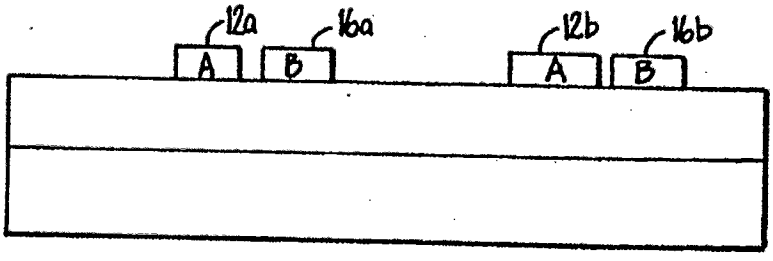


FIG._4.

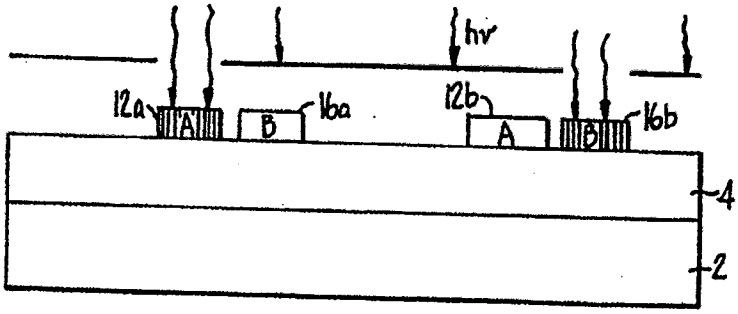
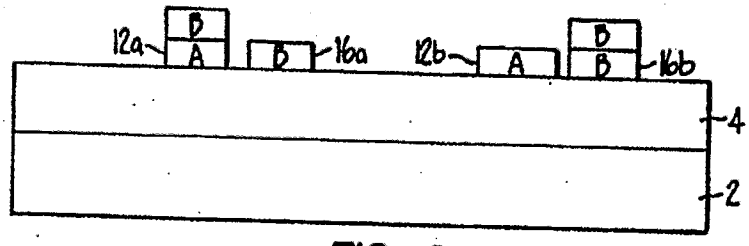


FIG._5.



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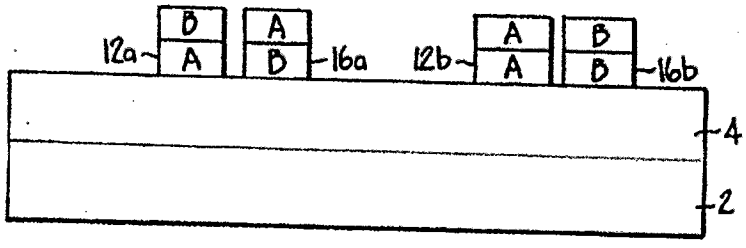

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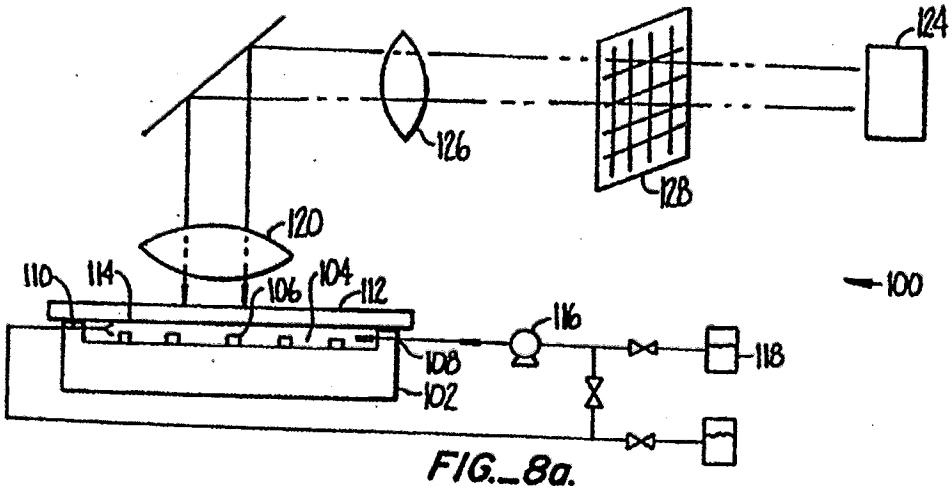
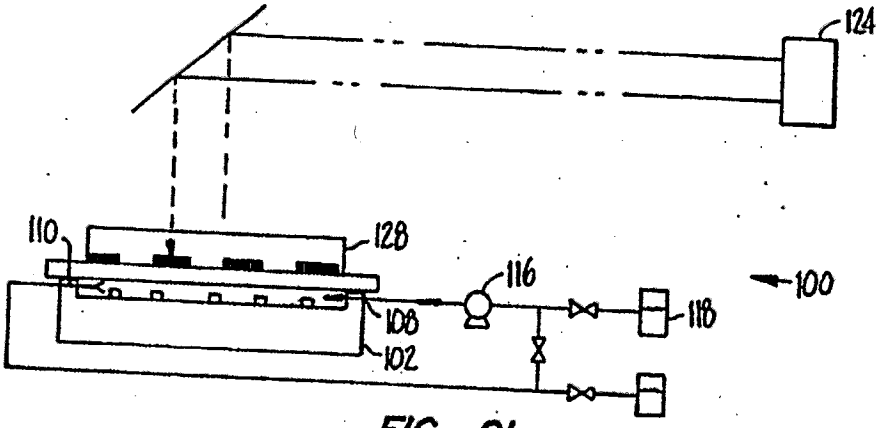


FIG._8a.



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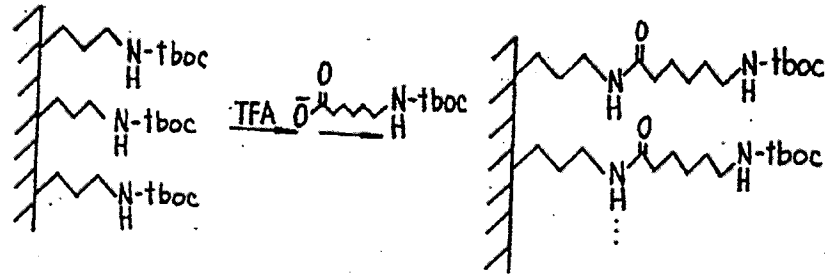
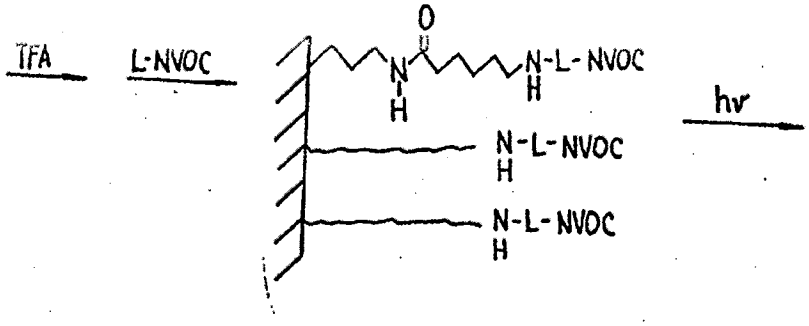




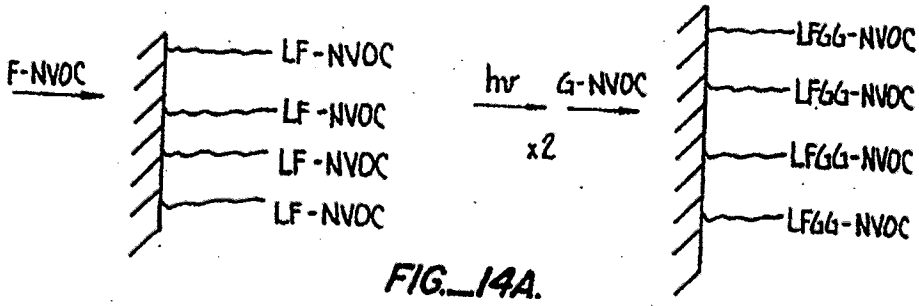
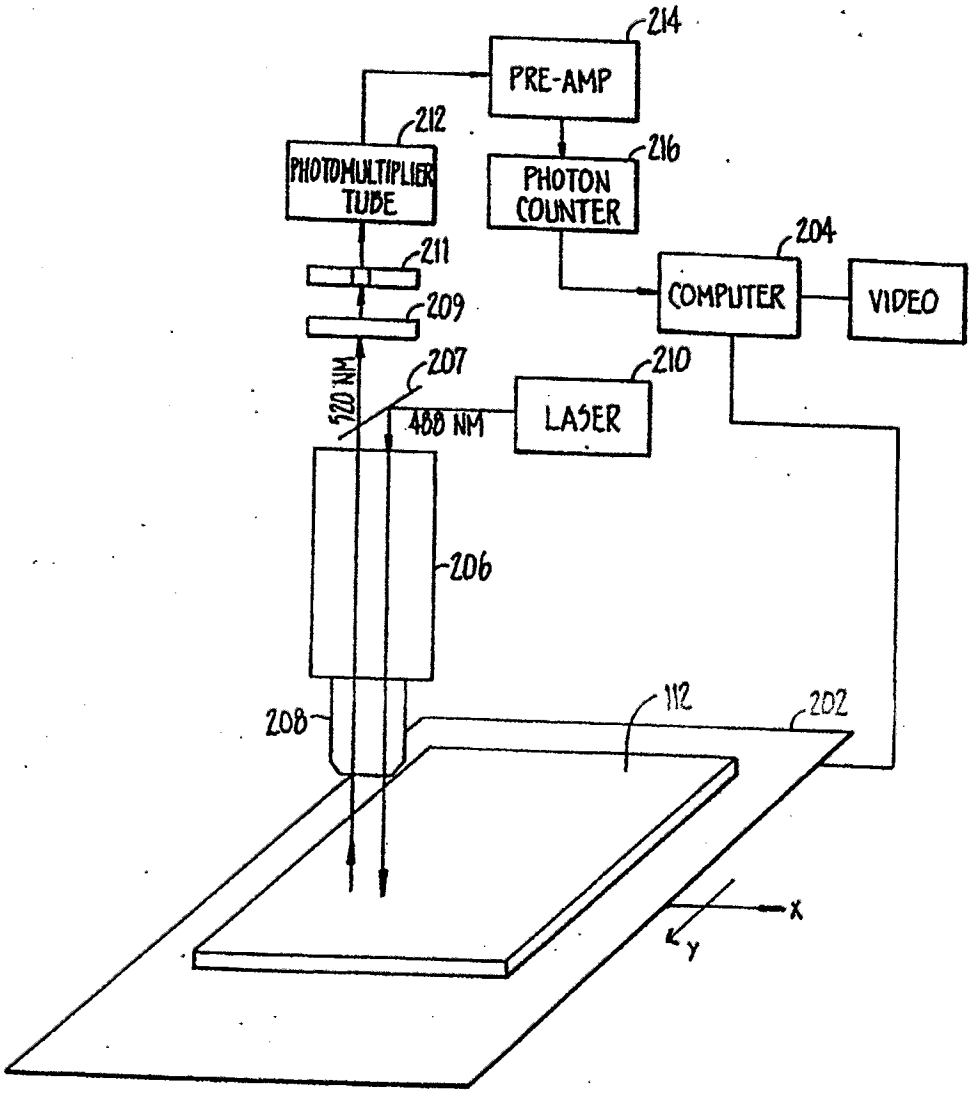


FIG._14A.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112
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X
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FIG. 9.

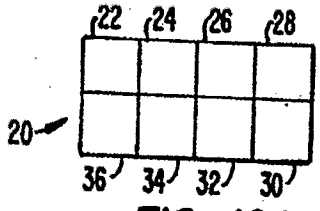
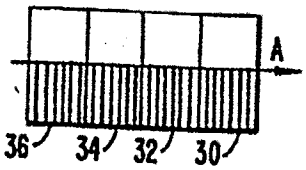


FIG._10A.



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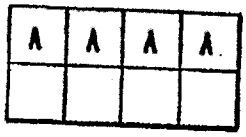


FIG._10C.

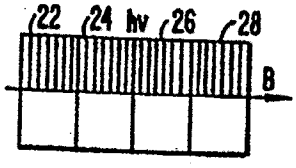


FIG._10D.

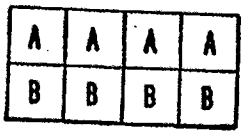


FIG._10E.

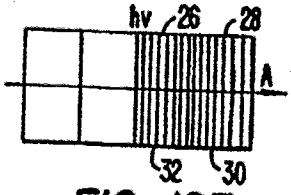


FIG._10F.

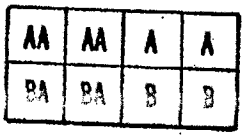


FIG._10G.

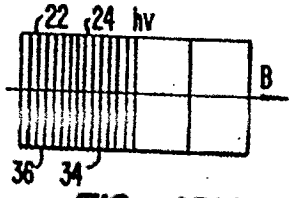


FIG._10H.

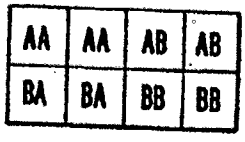


FIG._10I.

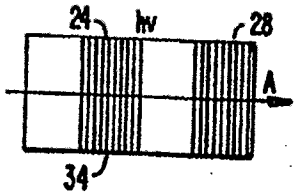


FIG._10J.

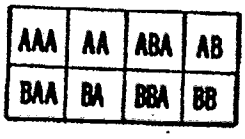


FIG._10K.

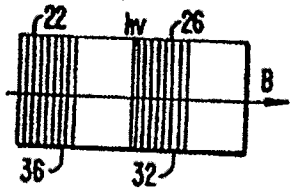


FIG._10L.

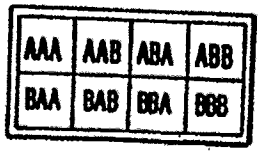


FIG._10M.

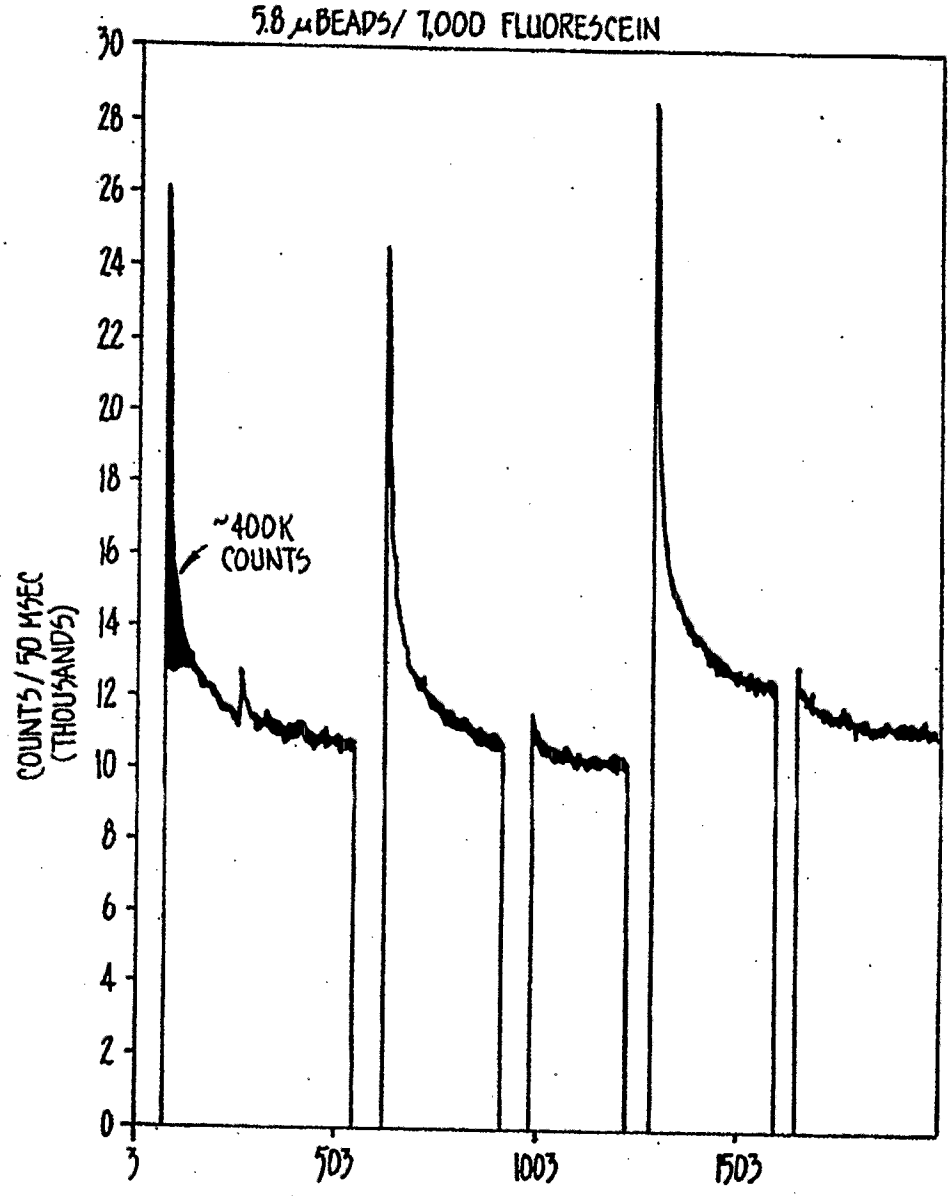

5.8 μBEADS/ 7,000 FLUORESCEIN
~400K COUNTS
COUNTS/50 MSEC (THOUSANDS)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
3
503
1003
1503
TIME (50 MSEC/PT)
~2 x 10⁻⁶ CHROMOPHORE/A²

FIG._11A.

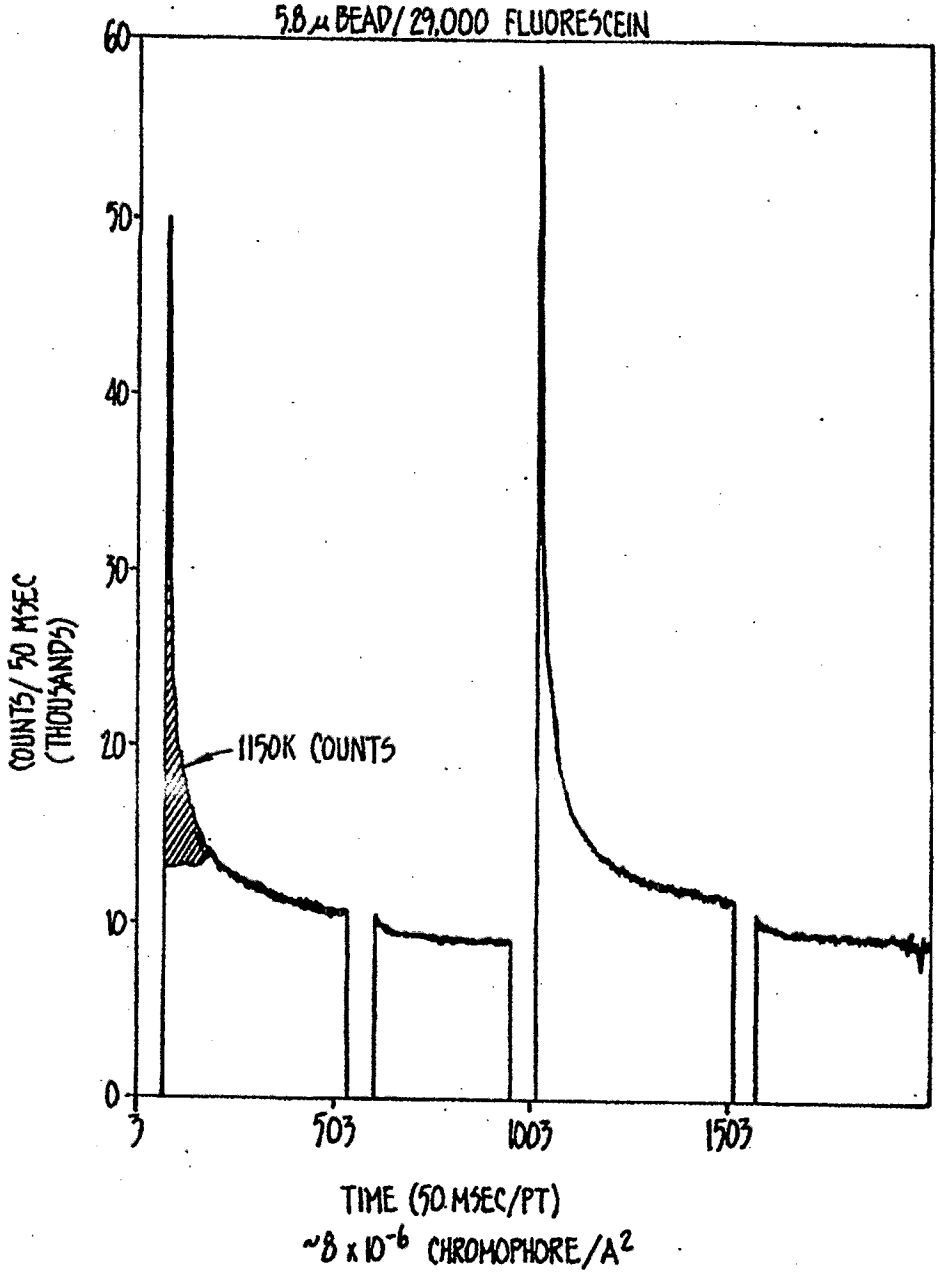

5.8μ BEAD/29,000 FLUORESCEIN
60
50
40
30
20
10
0
COUNTS/50 MSEC (THOUSANDS)
1150K COUNTS
3
503
1003
1503
TIME (50 MSEC/PT)
~8 x 10⁻⁶ CHROMOPHORE/A²

FIG. 11B.

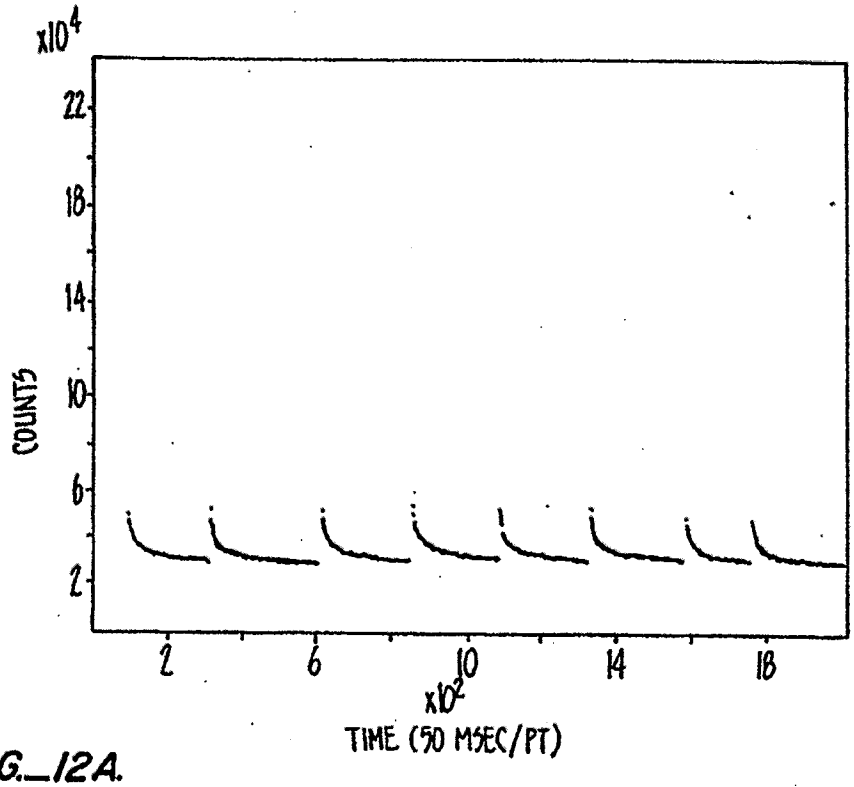


FIG._12A.

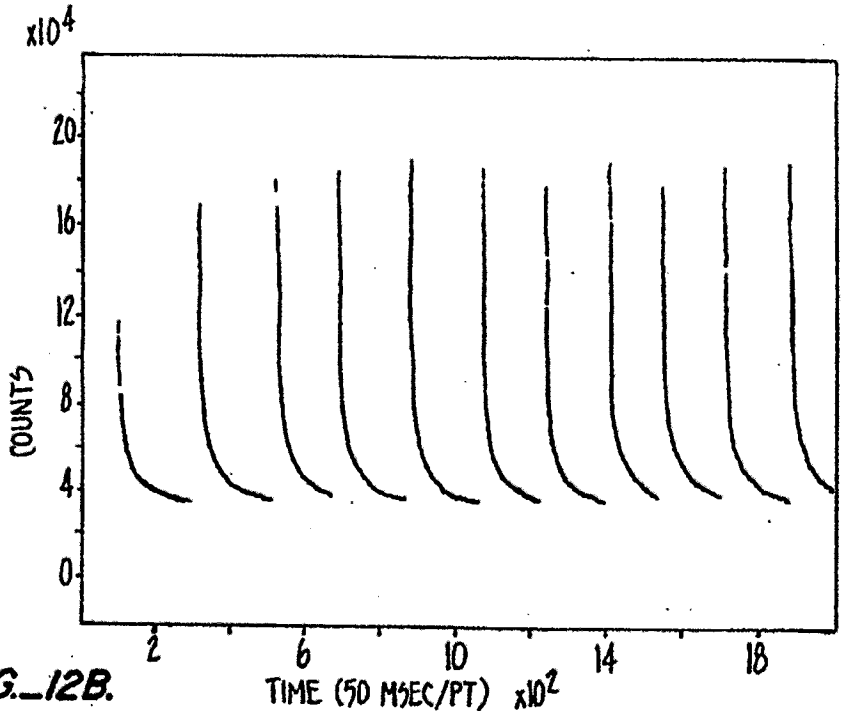


FIG._12B.

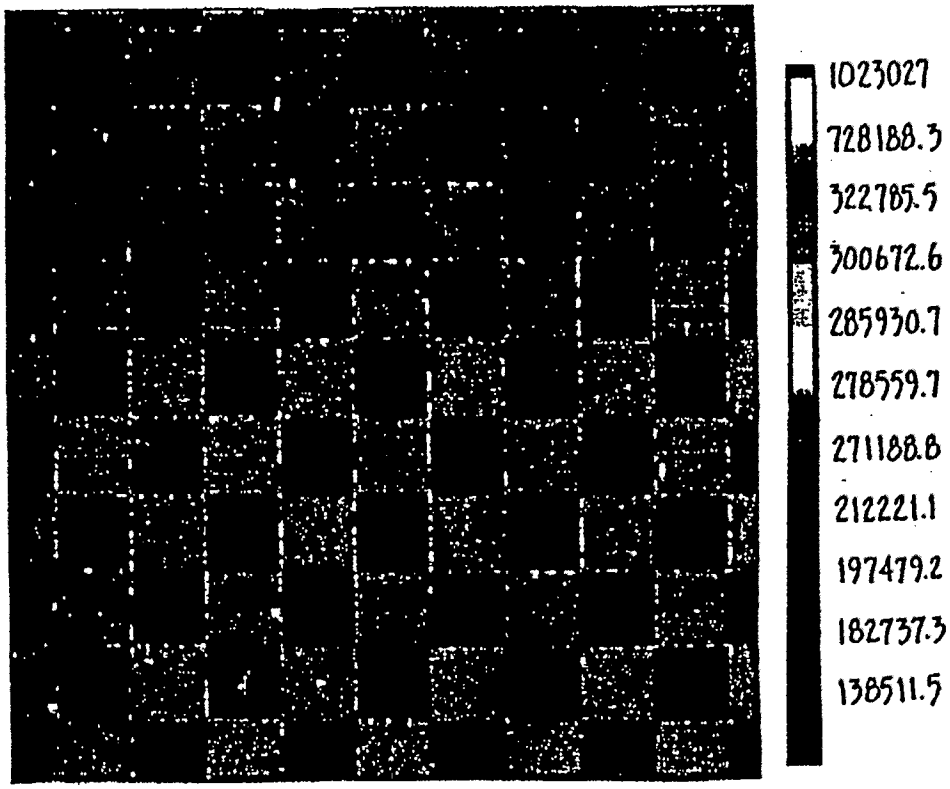

1023027
728188.3
322785.5
300672.6
285930.7
278559.7
271188.8
212221.1
197479.2
182737.3
138511.5
MEAN: 285930.7
VAR: 2.173242E+10
σ: 147419.2

FIG._13A.

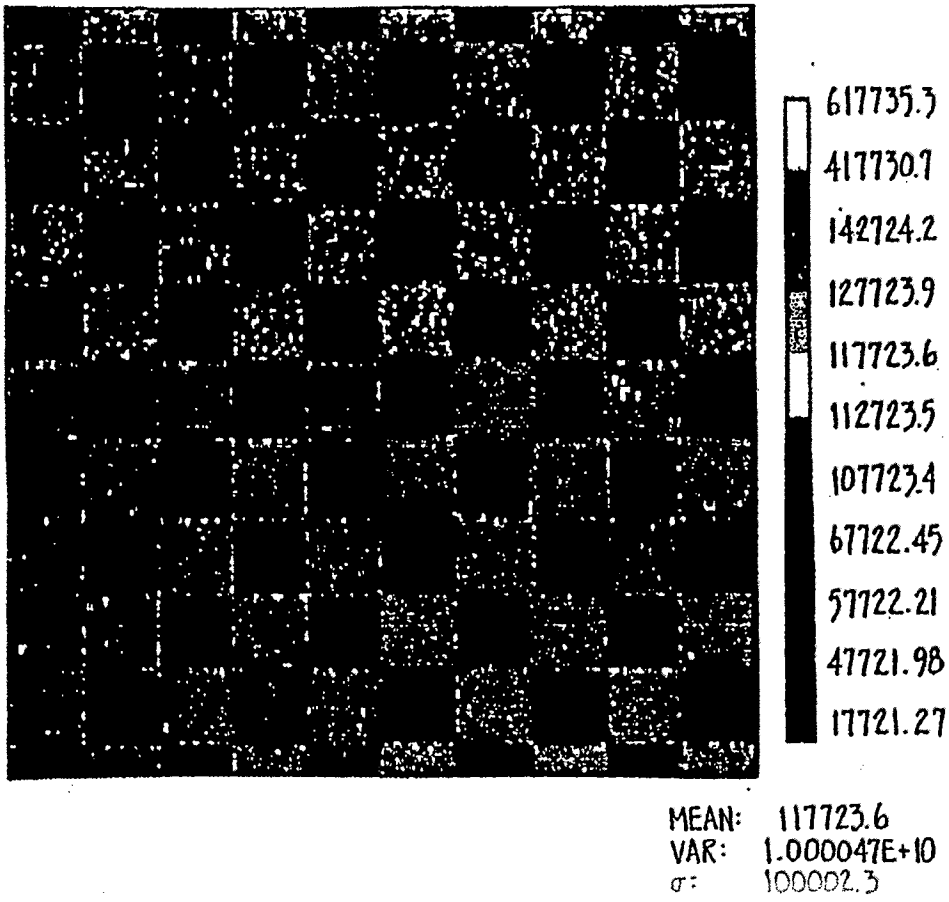

617735.3
417730.1
142724.2
127723.9
117723.6
112723.5
107723.4
67722.45
57722.21
47721.98
17721.27
MEAN: 117723.6
VAR: 1.000047E+10
σ: 100002.3

FIG._13B.

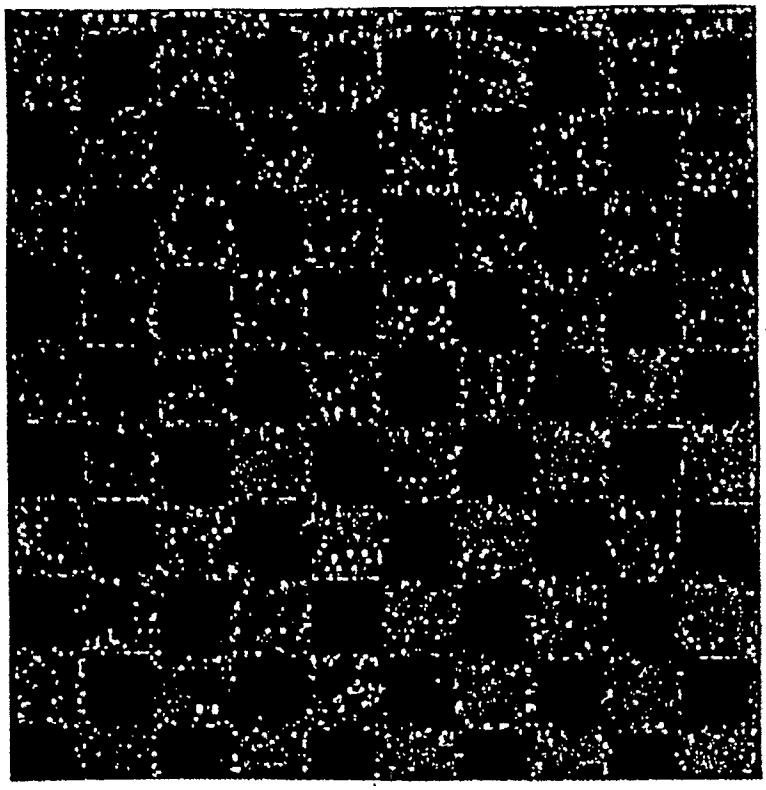

552484.3
313317.4
126963
113525.5
104567.2
100000
95608.83
59775.46
50017.12
41858.78
14983.75
MEAN: 104567.2
VAR: 8.025189E+09
σ: 89583.42

FIG._13C.

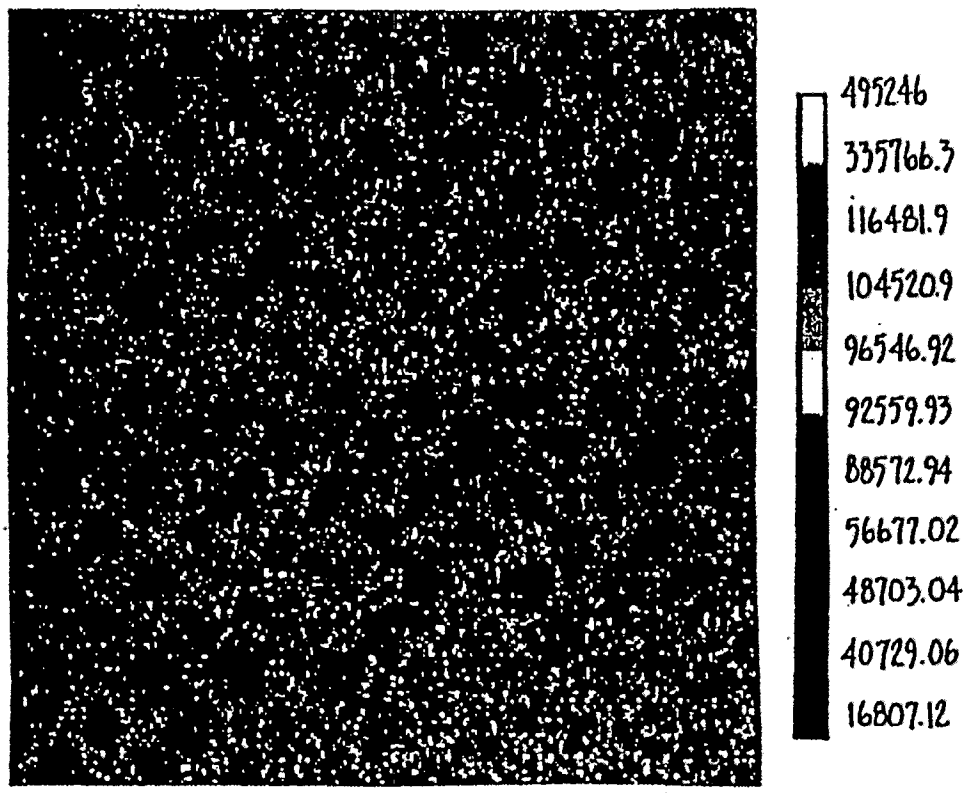

495246
335766.7
116481.9
104520.9
96546.92
92559.93
88572.94
56677.02
48703.04
40729.06
16807.12
MEAN: 96546.92
VAR: 6.358437E+09
σ: 79739.8

FIG._13D.

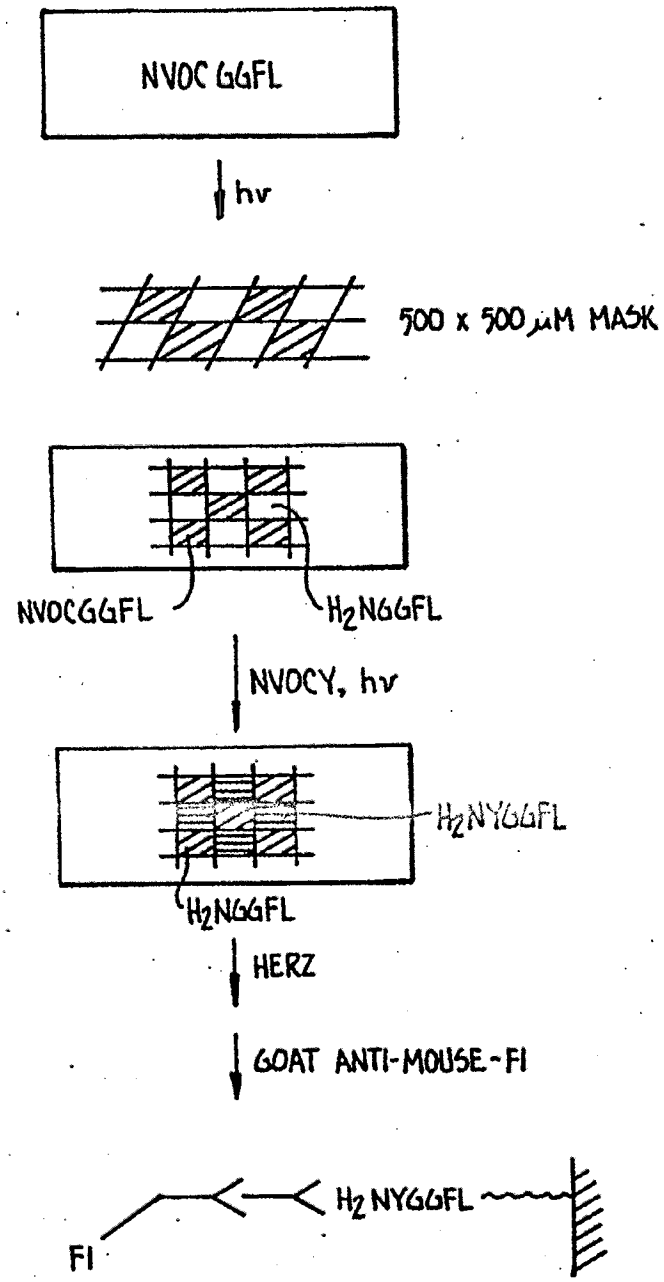

NVOC GGFL
hv
500 x 500 μM MASK
NVOCGGFL
H2NGGFL
NVOCY, hv
H2NYGGFL
H2NGGFL
HERZ
GOAT ANTI-MOUSE-FI
H2 NYGGFL
FI

FIG._14B.

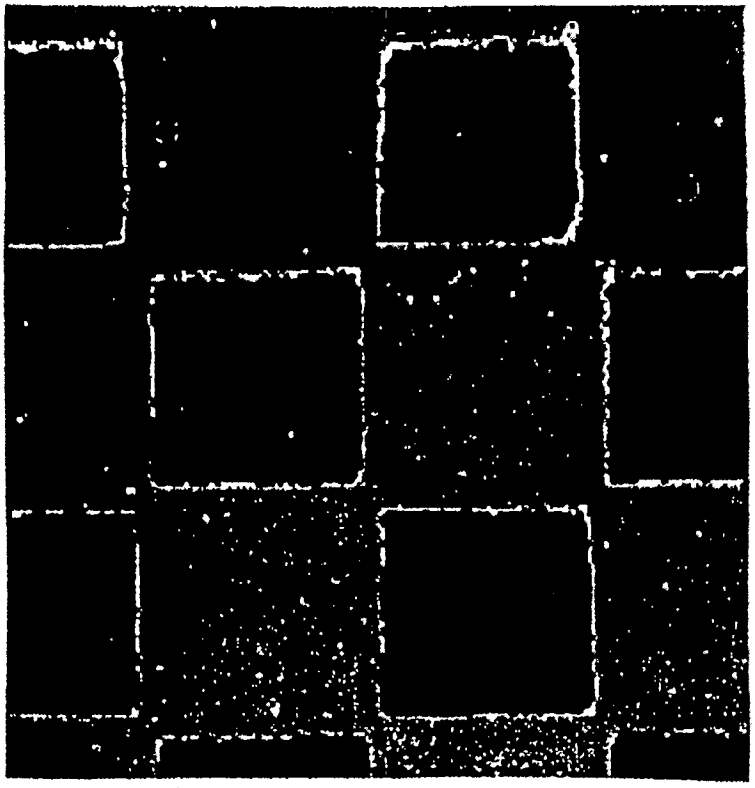
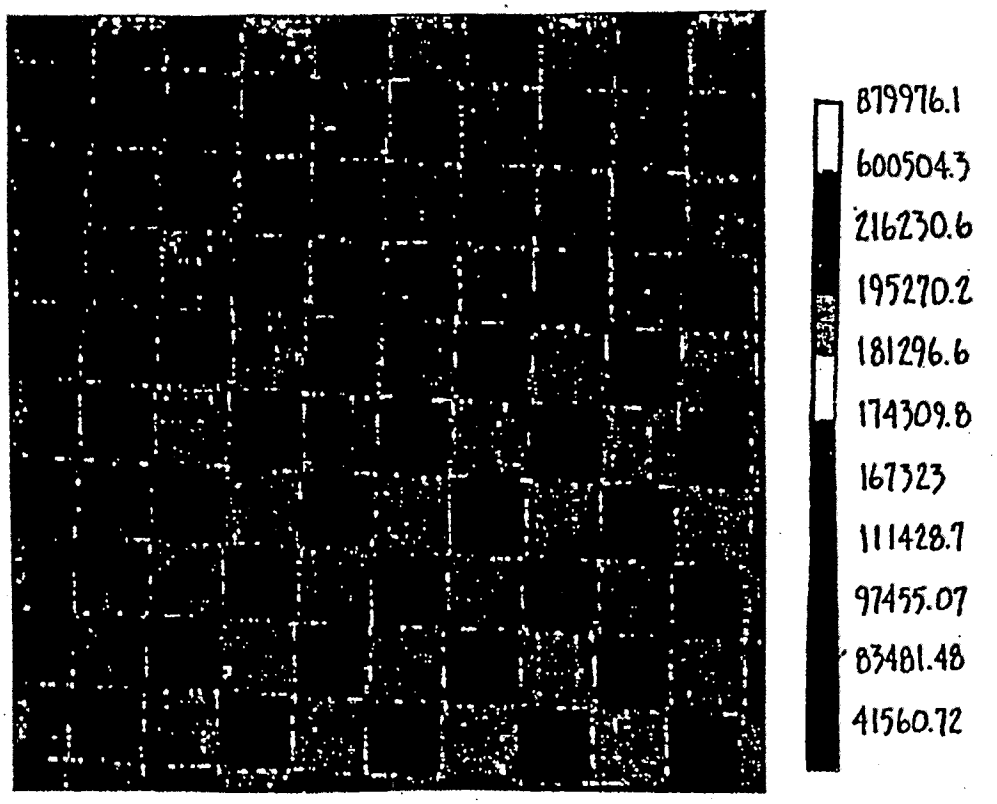

50780.26
34141.69
30813.97
28595.5
27486.26
26377.02
17503.12
11956.92
6410.734
-15774.03
37958.79
MEAN: 28595.5
VAR: 4.921637E+08
σ: 22184.76

FIG._15A.



MEAN: 181296.6
VAR: 1.952612E+10
σ: 139735.9

FIG._15B.

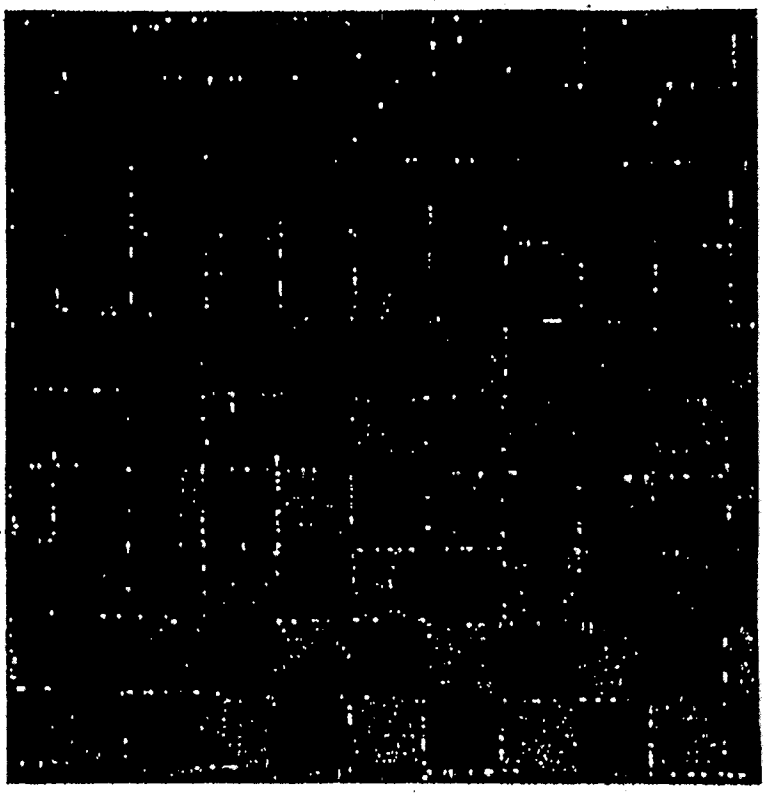
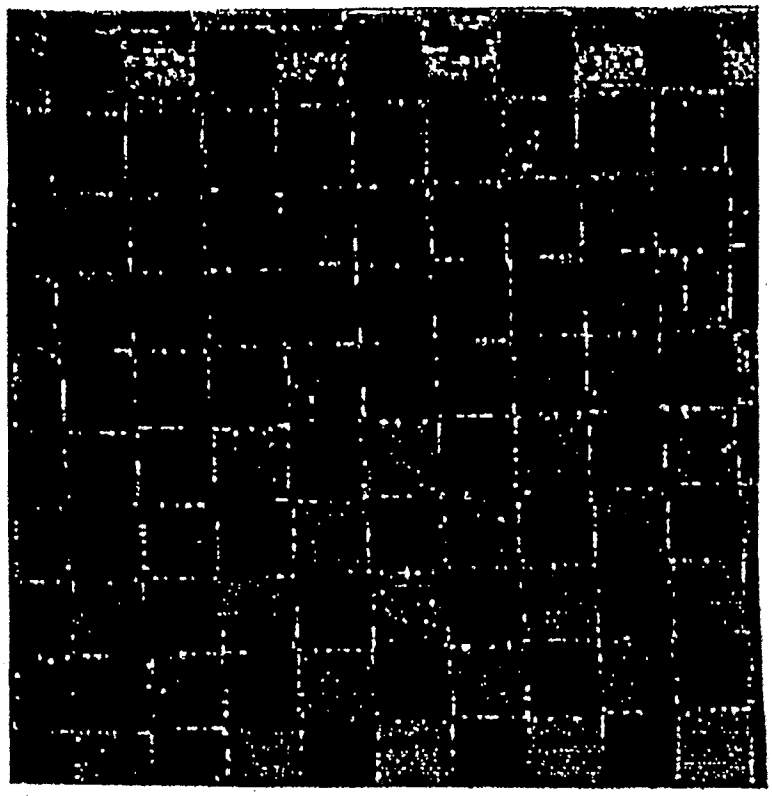

636588
428583.8
142577.9
126977.5
116577.3
111377.2
106177.1
64576.25
54176.03
43775.82
12575.18
MEAN: 116577.3
VAR: 1.081645E+10
σ: 104002.1

FIG._16.



MEAN: 131610.1
VAR: 1.148062E+10
σ: 107147.6

FIG._17.

P	A	S	G	
LPGFL	LAGFL	LSGFL	LGGFL	L
FPGFL	FAGFL	FSGFL	FGGFL	F
WPGFL	WAGFL	WSGFL	WGGFL	W
YPGFL	YAGFL	YSGFL	YGGFL	Y

L SET

FIG._18A.

P	a	s	G	
YpGFL	YaGFL	YsGFL	YGGFL	Y
fpGFL	faGFL	fsGFL	fGGFL	f
wpGFL	waGFL	wsGFL	wGGFL	w
YPGFL	yaGFL	ysGFL	yGGFL	y

D SET

FIG._18B.

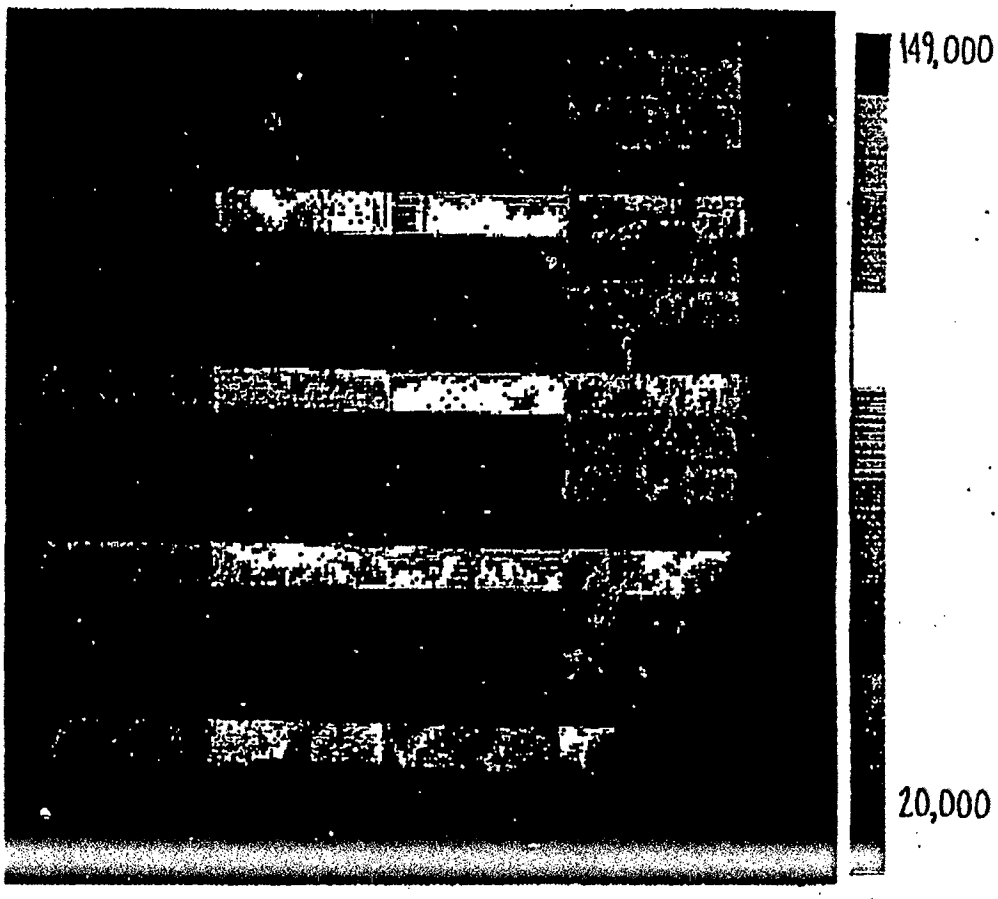


FIG._19.

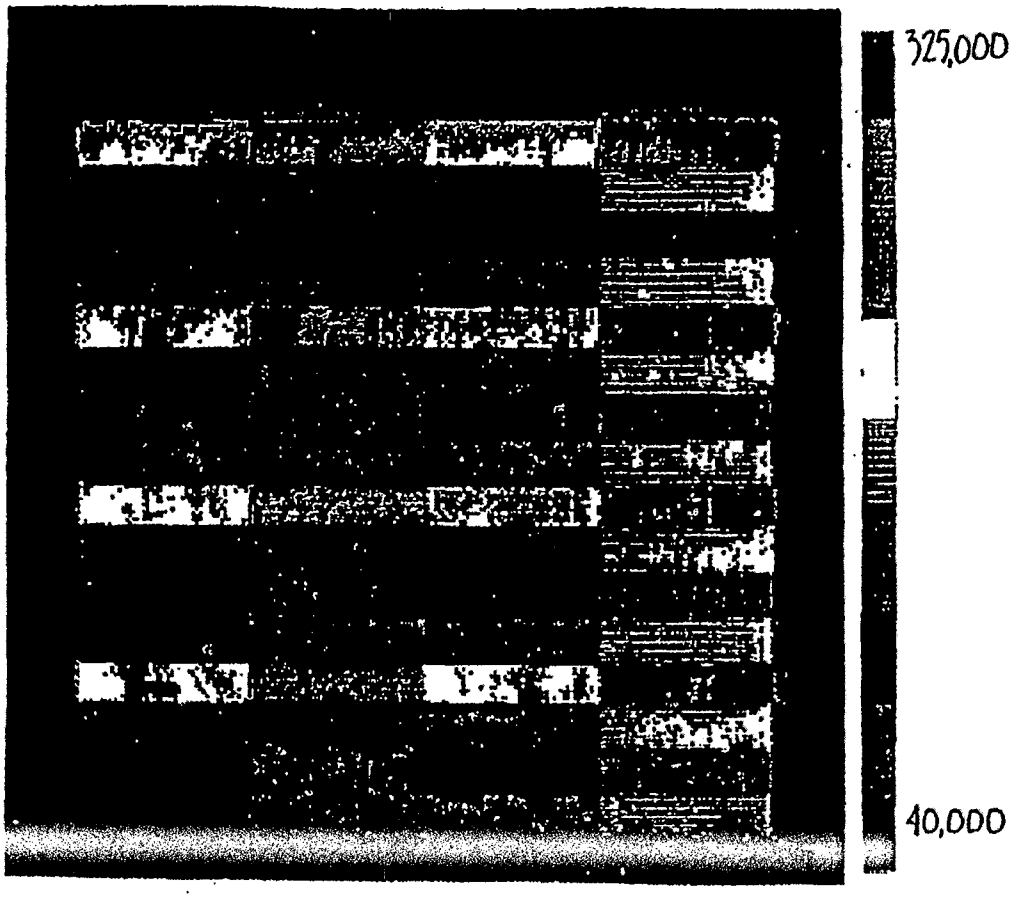


FIG._20.

LARGE SCALE PHOTOLITHOGRAPHIC SOLID PHASE SYNTHESIS OF POLYPEPTIDES AND RECEPTOR BINDING SCREENING THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of now abandoned application Ser. No. 362,901, filed Jun. 7, 1989 and assigned to the assignee of the present invention, now abandoned.

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

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BACKGROUND OF THE INVENTION

The present inventions relate to the synthesis and placement of materials at known locations. In particular, one embodiment of the inventions provides a method and associated apparatus for preparing diverse chemical sequences at known locations on a single substrate surface. The inventions may be applied, for example, in the field of preparation of oligomer, peptide, nucleic acid, oligosaccharide, phospholipid, polymer, or drug congener preparation, especially to create sources of chemical diversity for use in screening for biological activity.

The relationship between structure and activity of molecules is a fundamental issue in the study of biological systems. Structure-activity relationships are important in understanding, for example, the function of enzymes, the ways in which cells communicate with each other, as well as cellular control and feedback systems.

Certain macromolecules are known to interact and bind to other molecules having a very specific three-dimensional spatial and electronic distribution. Any large molecule having such specificity can be considered a receptor, whether it is an enzyme catalyzing hydrolysis of a metabolic intermediate, a cell-surface protein mediating membrane transport of ions, a glycoprotein serving to identify a particular cell to its neighbors, an IgG-class antibody circulating in the plasma, an oligonucleotide sequence of DNA in the nucleus, or the like. The various molecules which receptors selectively bind are known as ligands.

Many assays are available for measuring the binding affinity of known receptors and ligands, but the information which can be gained from such experiments is often limited by the number and type of ligands which are available. Novel ligands are sometimes discovered by chance or by application of new techniques for the elucidation of molecular structure, including x-ray crystallographic analysis and recombinant genetic techniques for proteins.

Small peptides are an exemplary system for exploring the relationship between structure and function in biology. A peptide is a sequence of amino acids. When the twenty naturally occurring amino acids are condensed into polymeric molecules they form a wide variety of three-dimensional configurations, each resulting from a particular amino acid sequence and solvent condition. The number of possible pentapeptides of the 20 naturally occurring amino acids, for example, is 20^5 or 3.2 million different peptides. The likelihood that molecules of this size might be useful in receptor-binding studies is supported by epitope analysis studies showing that some antibodies recognize sequences as short as a few amino acids with high specificity. Furthermore, the average molecular weight of amino acids puts small peptides in the size range of many currently useful pharmaceutical products.

Pharmaceutical drug discovery is one type of research which relies on such a study of structure-activity relationships. In most cases, contemporary pharmaceutical research can be described as the process of discovering novel ligands with desirable patterns of specificity for biologically important receptors. Another example is research to discover new compounds for use in agriculture, such as pesticides and herbicides.

Sometimes, the solution to a rational process of designing ligands is difficult or unyielding. Prior methods of preparing large numbers of different polymers have been painstakingly slow when used at a scale sufficient to permit effective rational or random screening. For example, the "Merrifield" method (*J. Am. Chem. Soc.* (1963) 85:2149–2154, which is incorporated herein by reference for all purposes) has been used to synthesize peptides on a solid support. In the Merrifield method, an amino acid is covalently bonded to a support made of an insoluble polymer. Another amino acid with an alpha protected group is reacted with the covalently bonded amino acid to form a dipeptide. After washing, the protective group is removed and a third amino acid with an alpha protective group is added to the dipeptide. This process is continued until a peptide of a desired length and sequence is obtained. Using the Merrifield method, it is not economically practical to synthesize more than a handful of peptide sequences in a day.

To synthesize larger numbers of polymer sequences, it has also been proposed to use a series of reaction vessels for polymer synthesis. For example, a tubular reactor system may be used to synthesize a linear polymer on a solid phase support by automated sequential addition of reagents. This method still does not enable the synthesis of a sufficiently large number of polymer sequences for effective economical screening.

Methods of preparing a plurality of polymer sequences are also known in which a porous container encloses a known quantity of reactive particles, the particles being larger in size than pores of the container. The containers may be selectively reacted with desired materials to synthesize desired sequences of product molecules. As with other methods known in the art, this method cannot practically be used to synthesize a sufficient variety of polypeptides for effective screening.

Other techniques have also been described. These methods include the synthesis of peptides on 96 plastic pins which fit the format of standard microtiter plates. Unfortunately, while these techniques have been somewhat useful, substantial problems remain. For example, these methods continue to be limited in the diversity of sequences which can be economically synthesized and screened.

From the above, it is seen that an improved method and apparatus for synthesizing a variety of chemical sequences at known locations is desired.

SUMMARY OF THE INVENTION

An improved method and apparatus for the preparation of a variety of polymers is disclosed.

In one preferred embodiment, linker molecules are provided on a substrate. A terminal end of the linker molecules is provided with a reactive functional group protected with a photoremovable protective group. Using lithographic methods, the photoremovable protective group is exposed to light and removed from the linker molecules in first selected regions. The substrate is then washed or otherwise contacted with a first monomer that reacts with exposed functional groups on the linker molecules. In a preferred embodiment, the monomer is an amino acid containing a photoremovable protective group at its amino or carboxy terminus and the linker molecule terminates in an amino or carboxy acid group bearing a photoremovable protective group.

A second set of selected regions is, thereafter, exposed to light and the photoremovable protective group on the linker molecule/protected amino acid is removed at the second set of regions. The substrate is then contacted with a second monomer containing a photoremovable protective group for reaction with exposed functional groups. This process is repeated to selectively apply monomers until polymers of a desired length and desired chemical sequence are obtained. Photolabile groups are then optionally removed and the sequence is, thereafter, optionally capped. Side chain protective groups, if present, are also removed.

By using the lithographic techniques disclosed herein, it is possible to direct light to relatively small and precisely known locations on the substrate. It is, therefore, possible to synthesize polymers of a known chemical sequence at known locations on the substrate.

The resulting substrate will have a variety of uses including, for example, screening large numbers of polymers for biological activity. To screen for biological activity, the substrate is exposed to one or more receptors such as antibodies, whole cells, receptors on vesicles, lipids, or any one of a variety of other receptors. The receptors are preferably labeled with, for example, a fluorescent marker, radioactive marker, or a labeled antibody reactive with the receptor. The location of the marker on the substrate is detected with, for example, photon detection or autoradiographic techniques. Through knowledge of the sequence of the material at the location where binding is detected, it is possible to quickly determine which sequence binds with the receptor and, therefore, the technique can be used to screen large numbers of peptides. Other possible applications of the inventions herein include diagnostics in which various antibodies for particular receptors would be placed on a substrate and, for example, blood sera would be screened for immune deficiencies. Still further applications include, for example, selective "doping" of organic materials in semiconductor devices, and the like.

In connection with one aspect of the invention an improved reactor system for synthesizing polymers is also disclosed. The reactor system includes a substrate mount which engages a substrate around a periphery thereof. The substrate mount provides for a reactor space between the substrate and the mount through or into which reaction fluids are pumped or flowed. A mask is placed on or focused on the substrate and illuminated so as to deprotect selected regions of the substrate in the reactor space. A monomer is pumped through the reactor space or otherwise contacted with the substrate and reacts with the deprotected regions. By selectively deprotecting regions on the substrate and flowing predetermined monomers through the reactor space, desired polymers at known locations may be synthesized.

Improved detection apparatus and methods are also disclosed. The detection method and apparatus utilize a substrate having a large variety of polymer sequences at known locations on a surface thereof. The substrate is exposed to a fluorescently labeled receptor which binds to one or more of the polymer sequences. The substrate is placed in a microscope detection apparatus for identification of locations where binding takes place. The microscope detection apparatus includes a monochromatic or polychromatic light source for directing light at the substrate, means for detecting fluoresced light from the substrate, and means for determining a location of the fluoresced light. The means for detecting light fluoresced on the substrate may in some embodiments include a photon counter. The means for determining a location of the fluoresced light may include an x/y translation table for the substrate. Translation of the slide and data collection are recorded and managed by an appropriately programmed digital computer.

A further understanding of the nature and advantages of the inventions herein may be realized by reference to the remaining portions of the specification and the attached drawings.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 illustrates masking and irradiation of a substrate at a first location. The substrate is shown in cross-section;

FIG. 2 illustrates the substrate after application of a monomer "A";

FIG. 3 illustrates irradiation of the substrate at a second location;

FIG. 4 illustrates the substrate after application of monomer "B";

FIG. 5 illustrates irradiation of the "A" monomer;

FIG. 6 illustrates the substrate after a second application of "B";

FIG. 7 illustrates a completed substrate;

FIGS. 8A and 8B illustrate alternative embodiments of a reactor system for forming a plurality of polymers on a substrate;

FIG. 9 illustrates a detection apparatus for locating fluorescent markers on the substrate;

FIGS. 10A–10M illustrate the method as it is applied to the production of the trimers of monomers "A" and "B";

FIGS. 11A, and 11B are fluorescence traces for standard fluorescent beads;

FIGS. 12A and 12B are fluorescence curves for NVOC (6-nitroveratiyloxycaronyl) slides not exposed and exposed to light respectively;

FIGS. 13A to 13D are fluorescence plots of slides exposed through 100 μm, 50 μm, 20 μm, and 10 μm masks;

FIGS. 14A and 14B illustrate formation of YGGFL (a peptide of sequence H_2N-tyrosine-glycine-glycine-phenylalanine-leucine-CO_2H) (a peptide of sequence H_2N-glycine-glycine-phenylalanine-leucine-CO_2H) followed by exposure to labeled Herz antibody (an antibody that recognizes YGGFL but not GGFL);

FIGS. 15A and 15B illustrate fluorescence plots of a slide with a checkerboard pattern of YGGFL and GGFL exposed to labeled Herz antibody.

FIG. 15A illustrates a 500×500 μm mask which has been focused on the substrate according to FIG. 8A while FIG. 15B illustrates a 50×50 μm mask placed in direct contact with the substrate in accord with FIG. 8B;

FIG. 16 is a fluorescence plot of YGGFL and PGGFL synthesized in a 50 μm checkerboard pattern;

FIG. 17 is a fluorescence plot of YPGGFL and YGGFL synthesized in a 50 μm checkerboard pattern;

FIGS. 18A and 18B illustrate the mapping of sixteen sequences synthesized on two different glass slides;

FIG. 19 is a fluorescence plot of the slide illustrated in FIG. 18A; and

FIG. 20 is a fluorescence plot of the slide illustrated in FIG. 10B.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

CONTENTS

I. Glossary
II. General
III. Polymer Synthesis
IV. Details of One Embodiment of a Reactor System
V. Details of One Embodiment of a Fluorescent Detection Device
VI. Determination of Relative Binding Strength of Receptors
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B Synthesis of Eight Trimers of "A" and "B"
C. Synthesis of a Dimer of an Aminopropyl Group and a Fluorescent Group
D. Demonstration of Signal Capability
E. Determination of the Number of Molecules Per Unit Area
F. Removal of NVOC and Attachment of a Fluorescent Marker
G. Use of a Mask
H. Attachment of YGGFL and Subsequent Exposure to Herz Antibody and Goat Antimouse
I. Monomer-by-Monomer Formation of YGGFL and Subsequent Exposure to Labeled Antibody
J. Monomer-by-Monomer Synthesis of YGGFL and PGGFL
K. Monomer-by-Monomer Synthesis of YGGFL and YPGGFL
L. Synthesis of an Array of Sixteen Different Amino Acid Sequences and Estimation of Relative Binding Affinity to Herz Antibody
VIII. Illustrative Alternative Embodiment
IX. Conclusion.

I. Glossary

The following terms are intended to have the following general meanings as they are used herein:

1. Complementary: Refers to the topological compatibility or matching together of interacting surfaces of a ligand molecule and its receptor. Thus, the receptor and its ligand can be described as complementary, and furthermore, the contact surface characteristics are complementary to each other.

2. Epitope: The portion of an antigen molecule which is delineated by the area of interaction with the subclass of receptors known as antibodies.

3. Ligand: A ligand is a molecule that is recognized by a particular receptor. Examples of ligands that can be investigated by this invention include, but are not restricted to, agonists and antagonists for cell membrane receptors, toxins and venoms, viral epitopes, hormones (e.g., steroids, etc.), hormone receptors, peptides, enzymes, enzyme substrates, cofactors, drugs (e.g., opiates, etc.) lectins, sugars, oligonucleotides, nucleic acids, oligosaccharides, proteins, and monoclonal antibodies.

4. Monomer: A member of the set of small molecules which can be joined together to form a polymer. The set of monomers includes but is not restricted to, for example, the set of common L-amino acids, the set of D-amino acids, the set of synthetic amino acids, the set of nucleotides and the set of pentoses and hexoses. As used herein, monomers refers to any member of a basis set for synthesis of a polymer. For example, dimers of L-amino acids form a basis set of 400 monomers for synthesis of polypeptides. Different basis sets of monomers may be used at successive steps in the synthesis of a polymer.

5. Peptide: A polymer in which the monomers are alpha amino acids and which are joined together through amide bonds and alternatively referred to as a polypeptide. In the context of this specification it should be appreciated that the amino acids may be the L-optical isomer or the D-optical isomer. Peptides are more than two amino acid monomers long, and often more than 20 amino acid monomers long. Standard abbreviations for amino acids are used (e.g., P for proline). These abbreviations are included in Stryer, *Biochemstry*, Third Ed., 1988, which is incorporated herein by reference for all purposes.

6. Radiation: Energy which may be selectively applied including energy having a wavelength of between 10^{-14} and 10^{4} meters including, for example, electron beam radiation, gamma radiation, x-ray radiation, ultraviolet radiation, visible light, infrared radiation, microwave radiation, and radio waves. "Irradiation" refers to the application of radiation to a surface.

7. Receptor: A molecule that has an affinity for a given ligand. Receptors may be naturally-occurring or manmade molecules. Also, they can be employed in their unaltered state or as aggregates with other species. Receptors may be attached, covalently or noncovalently, to a binding member, either directly or via a specific binding substance. Examples of receptors which can be employed by this invention include, but are not restricted to, antibodies, cell membrane receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants (such as on viruses, cells or other materials), drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles. Receptors are sometimes referred to in the art as anti-ligands. As the term receptors is used herein, no difference in meaning is intended. A "Ligand Receptor Pair" is formed when two macromolecules have combined through molecular recognition to form a complex.

Other examples of receptors which can be investigated by this invention include but are not restricted to:

a) Microorganism receptors: Determination of ligands which bind to receptors, such as specific transport proteins or enzymes essential to survival of microorganisms, is useful in a new class of antibiotics. Of particular value would be antibiotics against opportunistic fungi, protozoa, and those bacteria resistant to the antibiotics in current use.

b) Enzymes: For instance, the binding site of enzymes such as the enzymes responsible for cleaving neurotransmitters; determination of ligands which bind to certain receptors to modulate the action of the enzymes which cleave the different neurotransmitters is useful in the development of drugs which can be used in the treatment of disorders of neurotransmission.

c) Antibodies: For instance, the invention may be useful in investigating the ligand-binding site on the antibody molecule which combines with the epitope of an antigen of interest; determining a sequence that mimics an antigenic epitope may lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for auto-immune diseases (e.g., by blocking the binding of the "self" antibodies).

d) Nucleic Acids: Sequences of nucleic acids may be synthesized to establish DNA or RNA binding sequences.

e) Catalytic Polypeptides: Polymers, preferably polypeptides, which are capable of promoting a chemical reaction involving the conversion of one or more reactants to one or more products. Such polypeptides generally include a binding site specific for at least one reactant or reaction intermediate and an active functionality proximate to the binding site, which functionality is capable of chemically modifying the bound reactant. Catalytic polypeptides are described in, for example, copending U.S. application Ser. No. 404,920, which is incorporated herein by reference for all purposes.

f) Hormone receptors: For instance, the receptors for insulin and growth hormone. Determination of the ligands which bind with high affinity to a receptor is useful in the development of, for example, an oral replacement of the daily injections which diabetics must take to relieve the symptoms of diabetes, and in the other case, a replacement for the scarce human growth hormone which can only be obtained from cadavers or by recombinant DNA technology. Other examples are the vasoconstrictive hormone receptors; determination of those ligands which bind to a receptor may lead to the development of drugs to control blood pressure.

g) Opiate receptors: Determination of ligands which bind to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

8. Substrate: A material having a rigid or semi-rigid surface. In many embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches, or the like. According to other embodiments, small beads may be provided on the surface which may be released upon completion of the synthesis.

9. Protective Group: A material which is bound to a monomer unit and which may be spatially removed upon selective exposure to an activator such as electromagnetic radiation. Examples of protective groups with utility herein include Nitroveratryloxy carbonyl, Nitrobenzyloxy carbonyl, Dimethyl dimethoxybenzyloxy carbonyl, 5-Bromo-7-nitroindolinyl, o-Hydroxy-α-methyl cinnamoyl, and 2-Oxymethylene anthraquinone. Other examples of activators include ion beams, electric fields, magnetic fields, electron beams, x-ray, and the like.

10. Predefined Region: A predefined region is a localized area on a surface which is, was, or is intended to be activated for formation of a polymer. The predefined region may have any convenient shape, e.g., circular, rectangular, elliptical, wedge-shaped, etc. For the sake of brevity herein, "predefined regions" are sometimes referred to simply as "regions."

11. Substantially Pure: A polymer is considered to be "substantially pure" within a predefined region of a substrate when it exhibits characteristics that distinguish it from other predefined regions. Typically, purity will be measured in terms of biological activity or function as a result of uniform sequence. Such characteristics will typically be measured by way of binding with a selected ligand or receptor.

II. General

The present invention provides methods and apparatus for the preparation and use of a substrate having a plurality of polymer sequences in predefined regions. The invention is described herein primarily with regard to the preparation of molecules containing sequences of amino acids, but could readily be applied in the preparation of other polymers. Such polymers include, for example, both linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and peptides having either α-, β-, or ω-amino acids, heteropolymers in which a known drug is covalently bound to any of the above, polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, or other polymers which will be apparent upon review of this disclosure. In a preferred embodiment, the invention herein is used in the synthesis of peptides.

The prepared substrate may, for example, be used in screening a variety of polymers as ligands for binding with a receptor, although it will be apparent that the invention could be used for the synthesis of a receptor for binding with a ligand. The substrate disclosed herein will have a wide variety of other uses. Merely by way of example, the invention herein can be used in determining peptide and nucleic acid sequences which bind to proteins, finding sequence-specific binding drugs, identifying epitopes recognized by antibodies, and evaluation of a variety of drugs for clinical and diagnostic applications, as well as combinations of the above.

The invention preferably provides for the use of a substrate "S" with a surface. Linker molecules "L" are optionally provided on a surface of the substrate. The purpose of the linker molecules, in some embodiments, is to facilitate receptor recognition of the synthesized polymers.

Optionally, the linker molecules may be chemically protected for storage purposes. A chemical storage protective group such as t-BOC (t-butoxycarbonyl) may be used in some embodiments. Such chemical protective groups would be chemically removed upon exposure to, for example, acidic solution and would serve to protect the surface during storage and be removed prior to polymer preparation.

On the substrate or a distal end of the linker molecules, a functional group with a protective group P_0 is provided. The protective group P_0 may be removed upon exposure to radiation, electric fields, electric currents, or other activators to expose the functional group.

In a preferred embodiment, the radiation is ultraviolet (UV), infrared (IR), or visible light. As more fully described below, the protective group may alternatively

be an electrochemically-sensitive group which may be removed in the presence of an electric field. In still further alternative embodiments, ion beams, electron beams, or the like may be used for deprotection.

In some embodiments, the exposed regions and, therefore, the area upon which each distinct polymer sequence is synthesized are smaller than about 1 cm^2 or less than 1 mm^2. In preferred embodiments the exposed area is less than about 10,000 μm^2 or, more preferably, less than 100 μm^2 and may, in some embodiments, encompass the binding site for as few as a single molecule. Within these regions, each polymer is preferably synthesized in a substantially pure form.

Concurrently or after exposure of a known region of the substrate to light, the surface is contacted with a first monomer unit M_1 which reacts with the functional group which has been exposed by the deprotection step. The first monomer includes a protective group P_1. P_1 may or may not be the same as P_0.

Accordingly, after a first cycle, known first regions of the surface may comprise the sequence:

$$S—L—M_a—P_1$$

while remaining regions of the surface comprise the sequence:

$$S—L—P_0.$$

Thereafter, second regions of the surface (which may include the first region) are exposed to light and contacted with a second monomer M_2 (which may or may not be the same as M_1) having a protective group P_2. P_2 may or may not be the same as P_0 and P_1. After this second cycle, different regions of the substrate may comprise one or more of the following sequences:

$$S—L—M_1—M_2—P_2$$

$$S—L—M_2—P_2$$

$$S—L—M_1—P_1$$

and/or

$$S—L—P_0.$$

The above process is repeated until the substrate includes desired polymers of desired lengths. By controlling the locations of the substrate exposed to light and the reagents exposed to the substrate following exposure, the location of each sequence will be known.

Thereafter, the protective groups are removed from some or all of the substrate and the sequences are, optionally, capped with a capping unit C. The process results in a substrate having a surface with a plurality of polymers of the following general formula:

$$S—[L]—(M_i)—(M_j)—(M_k)\ldots(M_x)—[c]$$

where square brackets indicate optional groups, and $M_1 \ldots M_x$ indicates any sequence of monomers. The number of monomers could cover a wide variety of values, but in a preferred embodiment they will range from 2 to 100.

In some embodiments a plurality of locations on the substrate polymers are to contain a common monomer subsequence. For example, it may be desired to synthesize a sequence $S—M_1—M_2—M_3$ at first locations and a sequence $S—M_4—M_2—M_3$ at second locations. The process would commence with irradiation of the first locations followed by contacting with $M_1—P$, resulting in the sequence $S—M_1—P$ at the first location. The second locations would then be irradiated and contacted with $M_4—P$, resulting in the sequence $S—M_4—P$ at the second locations. Thereafter both the first and second locations would be irradiated and contacted with the dimer $M_2—M_3$, resulting in the sequence $S—M_1—M_2—M_3$ at the first locations and $S—M_4—M_2—M_3$ at the second locations. Of course, common subsequences of any length could be utilized including those in a range of 2 or more monomers, 2 to 100 monomers, 2 to 20 monomers, and a most preferred range of 2 to 3 monomers.

According to other embodiments, a set of masks is used for the first monomer layer and, thereafter, varied light wavelengths are used for selective deprotection. For example, in the process discussed above, first regions are first exposed through a mask and reacted with a first monomer having a first protective group P_1, which is removable upon exposure to a first wavelength of light (e.g., IR). Second regions are masked and reacted with a second monomer having a second protective group P_2, which is removable upon exposure to a second wavelength of light (e.g., UV). Thereafter, masks become unnecessary in the synthesis because the entire substrate may be exposed alternatively to the first and second wavelengths of light in the deprotection cycle.

The polymers prepared on a substrate according to the above methods will have a variety of uses including, for example, screening for biological activity. In such screening activities, the substrate containing the sequences is exposed to an unlabeled or labeled receptor such as an antibody, receptor on a cell, phospholipid vesicle, or any one of a variety of other receptors. In one preferred embodiment the polymers are exposed to a first, unlabeled receptor of interest and, thereafter, exposed to a labeled receptor-specific recognition element, which is, for example, an antibody. This process will provide signal amplification in the detection stage.

The receptor molecules may bind with one or more polymers on the substrate. The presence of the labeled receptor and, therefore, the presence of a sequence which binds with the receptor is detected in a preferred embodiment through the use of autoradiography, detection of fluorescence with a charge-coupled device, fluorescence microscopy, or the like. The sequence of the polymer at the locations where the receptor binding is detected may be used to determine all or part of a sequence which is complementary to the receptor.

Use of the invention herein is illustrated primarily with reference to screening for biological activity. The invention will, however, find many other uses. For example, the invention may be used in information storage (e.g., on optical disks), production of molecular electronic devices, production of stationary phases in separation sciences, production of dyes and brightening agents, photography, and in immobilization of cells, proteins, lectins, nucleic acids, polysaccharides and the like in patterns on a surface via molecular recognition of specific polymer sequences. By synthesizing the same compound in adjacent, progressively differing concentrations, a gradient will be established to control chemotaxis or to develop diagnostic dipsticks which, for example, titrate an antibody against an increasing amount